Levels of oxidative DNA damage are correlated with duration of Type 1 diabetes

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Introduction

Under conditions of oxidative stress damage can occur to all cellular biomolecules, including lipids, proteins, carbohydrates and DNA. Such damage has been implicated in the pathogenesis and long-term complications of Type I diabetes mellitus.

Aim

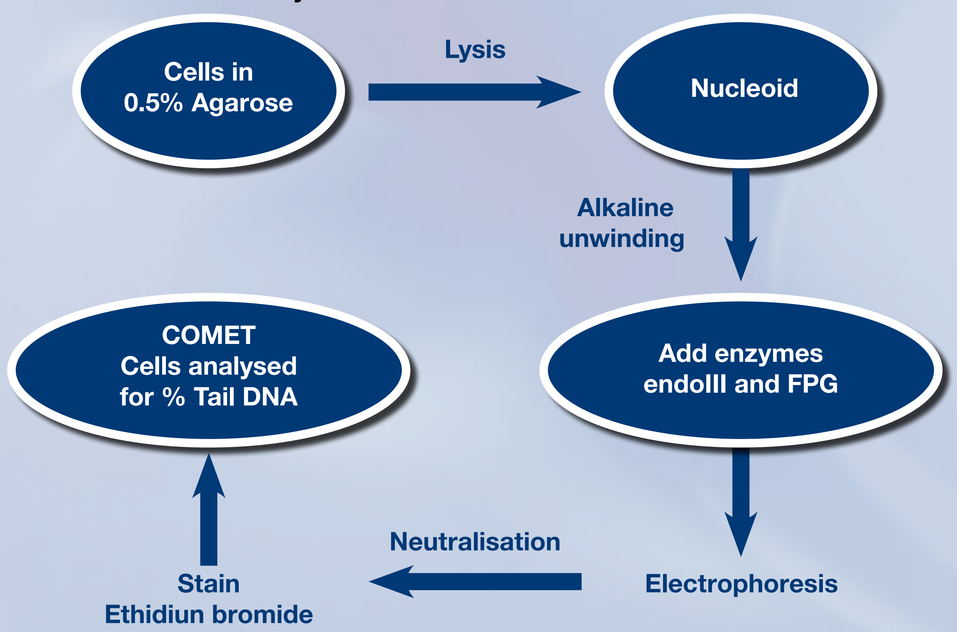
The aim of this study was to measure levels of oxidative DNA damage in peripheral blood lymphocytes in individuals with Type 1 diabetes, with relatively good glycaemic control, and to compare these with healthy age and gender matched control volunteers using a modification of the Alkaline Comet Assay.

Methods

Ethical Approval was obtained from Altnagelvin Area Hospital and the University of Ulster's Research Ethics Committee. Informed consent was obtained from 8 volunteers with Type 1 diabetes and 8ml of lithium heparin blood was taken by a nurse at the Diabetic Clinic. Eight age and gender matched healthy control volunteers also gave consent and blood samples were collected by a trained phlebotomist at the University of Ulster.

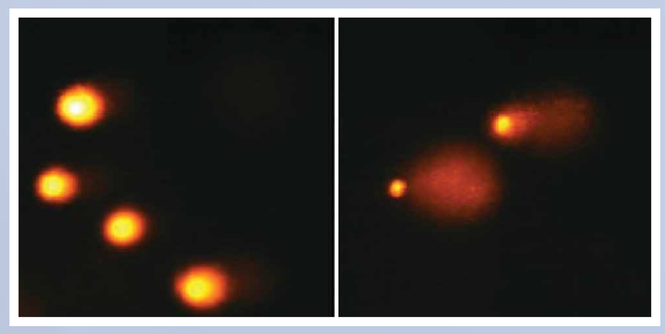
The modified Comet assay was used to measure levels of oxidative DNA damage in the lymphocytes from peripheral blood samples. The modified comet assay used was based on the original method of Singh et al. (1988) with an additional step involving repair endonucleases with appropriate lesion specificities endonuclease III (Endo III), for oxidised pyrimidines and formamidopyrimidine glycosylase (FPG) for ring-opened purines as well as 8-oxo-guanine (Collins et al. 1993). Glycated haemoglobin (HbA1c) was also measured in all volunteers'.

Figure 1: The Modified Comet Assay



Edno III = endonuclease III, FPG = formamidopyrimidine glycosylase

Figure 2: Image Analysis of "Comet Cells"



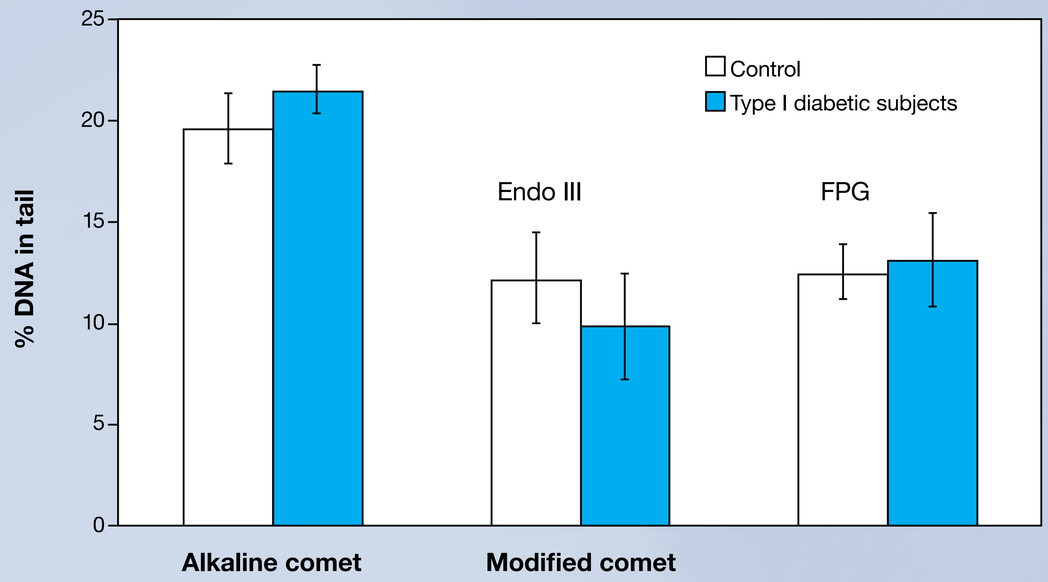
Lymphocytes stained with ethydium bromide.
Left: normal lymphocytes and right: damaged lymphocytes showing the characteristic Comet tail.

Results

The mean % HbA1c (Table 1) for the Type I diabetic subjects was 7.03 ± 0.10, which reflects good glycaemic control, as expected this was significantly higher than controls (4.58 ± 0.06; p <0.001).

The results failed to demonstrate a statistically significant difference in the mean levels of oxidative DNA damage in Type I diabetic subjects (mean endonuclease III 9.77 ± 2.6; mean formamidopyrimidine glycosylase 13.08 ± 2.3) compared to control subjects (mean endonuclease III 12.19 ± 2.2; mean formamidopyrimidine glycosylase 12.48 ± 1.4), see figure 3.

Figure 3: Levels of DNA damage (% DNA in tail) represented by the alkaline comet and levels of oxidative DNA damage (% DNA in tail) represented by Endo III sensitive sites and FPG sensitive sites from Type I diabetic and control subjects.



However linear regression analysis revealed a statistically significant (p = 0.024) positive correlation between the number of formamidopyrimidine glycosylase sensitive-sites and duration of Type I diabetes mellitus. In addition a positive correlation was observed between the number of endonuclease III sensitive-sites and duration of Type I diabetes mellitus, though this was not significant (p = 0.078).

Conclusions

These results indicate that even with good glycaemic control there is a positive correlation between levels of oxidative DNA damage and duration of Type I diabetes in vivo. The physiological effect(s) of this DNA damage remain to be elucidated.

References

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Singh NP, Mc Coy MT, Trice RR & Schneider EL (1988) A simple technique for quantification of low levels of DNA damage in individual cells. *Experimental Cell Research* **175**, 184-191.

Acknowledgements

The enzymes were kindly supplied by Prof A.R. Collins (Rowett Research Institute, Aberdeen, Scotland, UK